

FcεRI engagement of Langerhans cell-like dendritic cells and inflammatory dendritic epidermal cell-like dendritic cells induces chemotactic signals and different T-cell phenotypes in vitro

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Background: Atopic dermatitis (AD) is a biphasic inflammatory skin disease characterized by an initial phase predominated by T_H2 cytokines, which switches into a second T_H1-dominated chronic phase. Thus far, the small number of FcεRI-bearing Langerhans cells (LCs) and inflammatory dendritic epidermal cells (IDECs) in the epidermis of patients with AD has hampered a detailed functional analysis and limited our knowledge of these dendritic cells (DCs).

Objective: We studied FcεRI-mediated mechanisms of LCs and IDECs with the help of a novel in vitro model.

Methods: Langerhans cell-like dendritic cells (LC-DCs) and inflammatory dendritic epidermal cell-like dendritic cells (IDEC-DCs) bearing FcεRI have been generated from monocytes of the same atopic donor and compared functionally with LCs and IDECs isolated from the skin of patients with AD.

Results: We found that FcεRI-activated LC-DCs release chemotactic signals, and supernatants of FcεRI-activated LC-DCs increase the migratory capacity of precursor cells of IDECs and naive T cells in vitro. FcεRI-activated IDEC-DCs produce high amounts of proinflammatory cytokines and chemokines and might thereby amplify the inflammatory immune reaction in patients with AD. Furthermore, FcεRI-activated IDEC-DCs prime naive T cells into IFN-γ-producing T cells and release IL-12 and IL-18, which together might lead to the switch of the initial T_H2-type immune response into a response of the T_H1 type in vivo.

Conclusion: The present study provides evidence that FcεRI-activated LC-DCs and IDEC-DCs contribute distinctly to the outcome of T-cell responses in vitro and might have implications for the biphasic nature of AD in vivo. (J Allergy Clin Immunol 2004;113:949-57.)

Key words: Dendritic cells, antigen presentation, IgE, skin, inflammation

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Abbreviations used

AD:	Atopic dermatitis
DC:	Dendritic cell
IDEC:	Inflammatory dendritic epidermal cell
IDEC-DC:	Inflammatory dendritic epidermal cell-like dendritic cell
LC:	Langerhans cell
LC-DC:	Langerhans cell-like dendritic cell
MCP-1:	Macrophage chemoattractant protein 1
MFI:	Mean fluorescence intensity
RFI:	Relative fluorescence intensity
TCC:	T-cell clone

Atopic dermatitis (AD) is a chronic relapsing skin disease associated with an abnormal activation and proliferation of distinct subpopulations of helper T lymphocytes (T cells) reactive to allergens that are presented by dendritic cell (DC) subtypes. The initial phase of this biphasic disease is predominated by T cells producing cytokines of the T_H2 type, such as IL-4. Later, this T_H2 cytokine pattern switches into the second phase, which is predominated by the T_H1 cytokine IFN-γ, leading to chronic skin lesions.¹

One characteristic of AD is the prominent skin infiltration with hyperstimulatory DCs.² Classical epidermal Langerhans cells (LCs) are characterized by their cytoplasmic Birbeck granules and their CD1a/CD207-positive phenotype.³ In contrast, inflammatory dendritic epidermal cells (IDECs) lack the typical Birbeck granules but express the mannose receptor (CD206).^{4,5} A hallmark of both epidermal LCs and IDECs in the skin lesions of patients with AD is the increased expression of FcεRI.⁶⁻⁸ It is assumed that allergens that penetrate the epidermis through the reduced skin barrier of patients with AD⁹ are taken up by FcεRI-bound IgE molecules of epidermal DCs.^{10,11} This leads to an efficient antigen presentation to T cells and later to the polarization of naive T cells into T_H1 or T_H2 cells. Recently, it has been shown that allergen challenge to the skin in atopy patch test reactions induces the rapid invasion of IDECs into the epidermis and the development of eczematous skin lesions in patients with AD.¹²

Because of the small number of ex vivo isolated LCs and IDECs available and the lack of a reliable animal

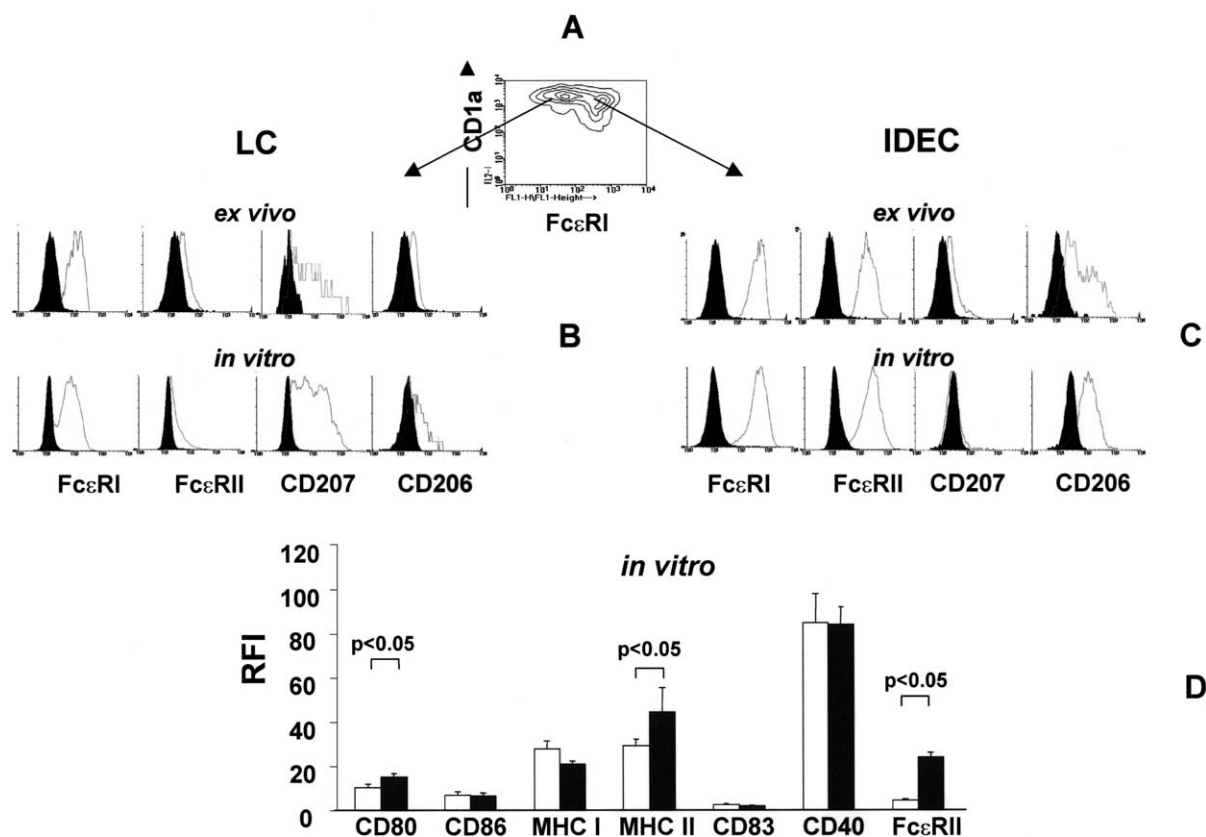


FIG 1. LC-DCs and IDEC-DCs generated in vitro are similar to LCs and IDECs in vivo. **A**, Two distinct FcεRI-bearing (x-axis) CD1a⁺ DC subtypes (y-axis) can be differentiated in this dot-plot histogram. LCs (**B**) or IDECs (**C**) isolated ex vivo and generated in vitro are shown. These data show one representative experiment (n = 5). **D**, Quantitative differences of the surface expression of costimulatory molecules and MHC molecules of LC-DCs (open bars) and IDEC-DCs (filled bars) are shown. The relative fluorescence index (RFI) of the expression of each marker is indicated on the y-axis (n = 12).

model, detailed information about the respective role of the 2 DC types is missing. Therefore it was important to establish an in vitro model that allows the generation of Langerhans cell-like DCs (LC-DCs)¹³ and inflammatory dendritic epidermal cell-like DCs (IDEC-DCs)¹⁴ from monocytes of the same atopic donor bearing functional FcεRI, which display phenotypic characteristics of LCs and IDECs in vivo. This enables us for the first time to study FcεRI-positive LC- and IDEC-mediated mechanisms and their contribution to the pathomechanisms of AD.

METHODS

For information on reagents, please see the ancillary material in the Journal's Online Repository (available at www.jaci.com/jaci).

Cell preparation and culture

Shave biopsy specimens were taken from the patients with AD according to the criteria of Hanifin and Rajka after obtaining signed informed consent in accordance with the approval of the local ethics committee.¹⁵ Seventeen skin lesions from nonlesional skin harboring LCs and lesional skin harboring LCs and IDECs were sampled and prepared from skin biopsy specimens by means of trypsinization, as described previously.¹⁶ Blood samples from patients with AD (n = 30) were obtained, as described in detail elsewhere.^{14,18} LC-DCs

were generated by culturing monocytes for 8 days in medium containing 500 IU/mL human recombinant GM-CSF (10 ng/mL; 500 IU/mL; Leucomax, Novartis Pharma AG) human recombinant IL-4 (Gibco) and natural human TGF-β1 (10 ng/mL; 25 U/mL; R&D systems), as described previously,¹⁸ and IDEC-DCs with GM-CSF (50 ng/mL, 500 IU/mL), recombinant IL-4 (10 ng/mL, 500 IU/mL), and the reducing agent 2-mercaptoethanol (2-ME; 50 μmol/L) in 24-well plastic plates (Corning).¹⁴

For information on isolation of naive and memory T cells, please see the ancillary material in the Journal's Online Repository (available at www.mosby.com/jaci).

Flow cytometric analysis

Immunolabeling for phenotyping was performed as previously reported.^{19,20} Intracellular staining for cytokines and chemokines was carried out according to the manufacturer's instructions (Pharmingen). After washing with PBS, the cells were analyzed with a FACS Calibur (Becton Dickinson), as described in detail elsewhere.¹⁹ As a control, cells were also stained with a corresponding phycoerythrin-labeled isotype-matched control immunoglobulin. All incubations and washes were performed at 4°C. Results were expressed as the percentage of positive cells and relative fluorescence intensity (RFI) calculated from mean fluorescence intensity (MFI) as follows:

$$RFI = \frac{MFI_{\text{parameter evaluated}} - MFI_{\text{control}}}{MFI_{\text{control}}}$$

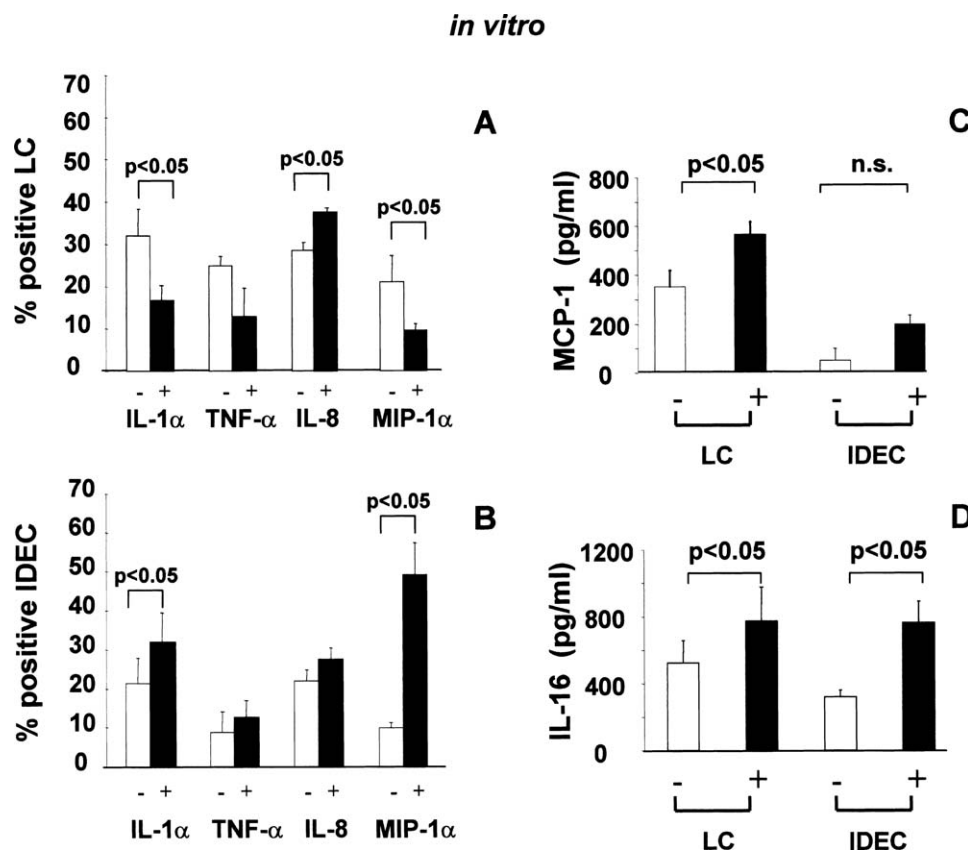


FIG 2. IDEC-DCs increase their capacity to produce proinflammatory cytokines and chemokines after Fc ϵ RI ligation *in vitro*. The percentage of the intracellular amount of proinflammatory cytokines of unstimulated (-) and Fc ϵ RI-stimulated LC-DCs (+; **A**) and unstimulated (-) and stimulated IDEC-DCs (+; **B**) is indicated on the y-axis (n = 5). The amount of MCP-1 (**C**) and IL-16 (**D**) released by Fc ϵ RI-stimulated (+) and unstimulated (-) LC-DCs and IDEC-DCs is indicated on the y-axis (in picograms per milliliter; n = 8).

IgE-dependent activation of LC-DCs and IDEC-DCs

LC-DCs and IDEC-DCs were washed and incubated at 2.5×10^5 cells/mL in 24-well flat-bottom culture plates with purified monoclonal human IgE specific for the major birch pollen allergen Bet v 1 (1 μ g/mL) for 1 hour at 37°C. After a second wash step, LC-DCs and IDEC-DCs were incubated with purified recombinant Bet v 1 (10 μ g/mL). As a control, the cells were incubated with either monoclonal human Bet v 1-specific IgE or Bet v 1 alone. Cross-linking of Fc ϵ RI cells with human IgE and anti-human IgE and the respective control conditions for the cytokine and chemokine assays was achieved as described previously.¹⁹

Detection of cytokines in the cell culture supernatant

For ELISA, the supernatants were stored at -70°C until the cytokine measurements were performed. Quantification of cytokines released from Fc ϵ RI-stimulated or Fc ϵ RI-unstimulated LC-DCs and IDEC-LCs in the supernatants was carried out in triplicate with an ELISA Kit (PBL Biomedical Laboratories), according to the manufacturer's instructions.

For information on T-cell proliferation assays, please see the ancillary material in the Journal's Online Repository (available at www.mosby.com/jaci).

Polarization of naive T cells

Fc ϵ RI on LC-DCs and IDEC-DCs was loaded with Bet v 1-specific chimeric IgE (1 μ g/mL), washed and loaded with recombinant Bet v 1 (10 μ g/mL), irradiated (3000 Gy), and cocultured with autologous naive CD4/CD45RA-positive T cells to assess cytokine secretion by T cells after activation by LC-DCs and IDEC-DCs and to detect *in situ* cytokine production. T cells were then stimulated with 50 ng/mL phorbol 12-myristate 13-acetate (Sigma) plus 500 ng/mL ionomycin (Sigma) for 6 hours. Monensin (2 μ mol/L; Sigma) was added during the final 2 hours. Cells were then stained with mAb against CD3 (peridinin chlorophyll A protein) and fixed with 1% paraformaldehyde in PBS, permeabilized with saponin, and stained with mAb against IL-4 (phycoerythrin) and IFN- γ (FITC).

For information on chemotaxis assay, please see the ancillary material in the Journal's Online Repository (available at www.mosby.com/jaci).

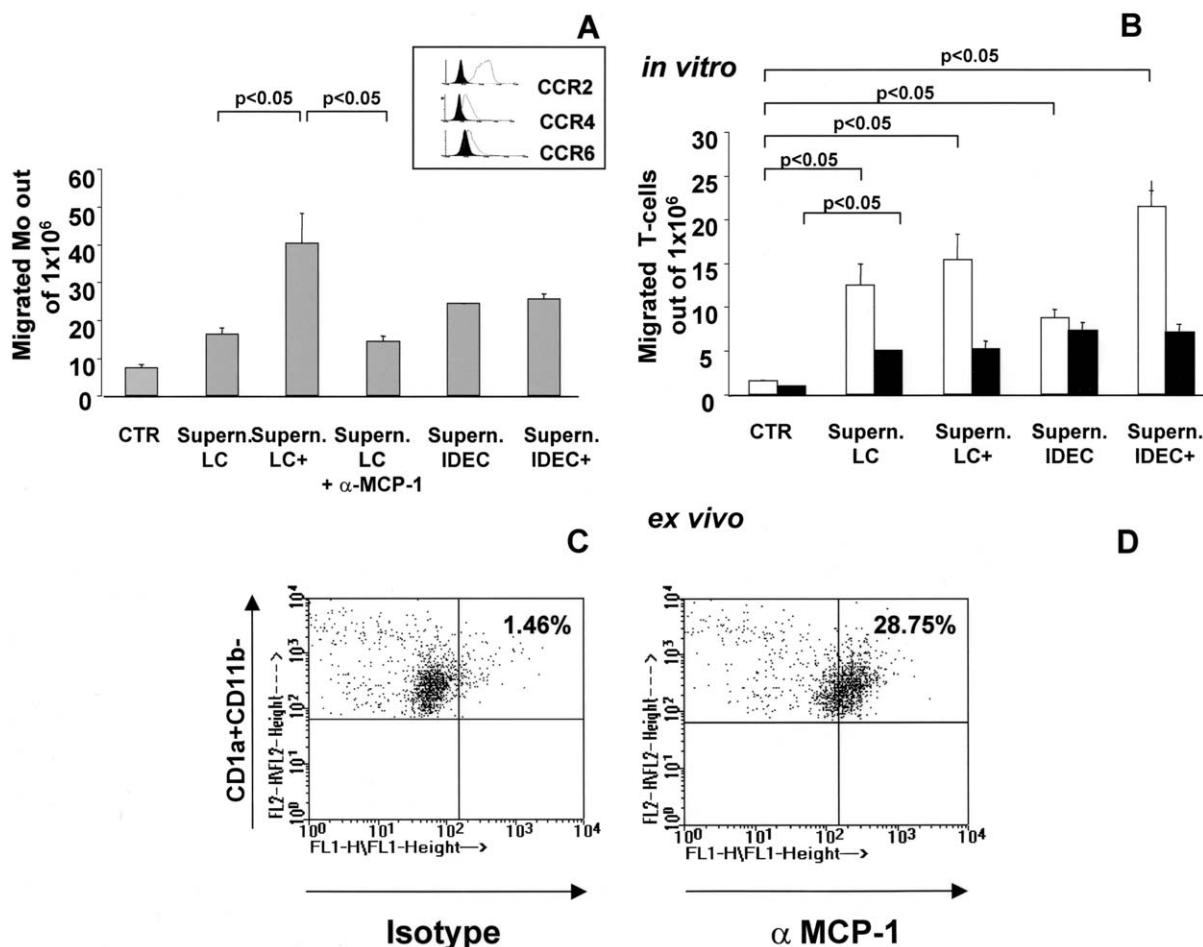


FIG 3. MCP-1 in the supernatant of Fc ϵ RI-stimulated LC-DCs induces an increased migration of monocytes from the peripheral blood in vitro. Monocytes display a high expression of CCR2 (**A**). Incubation of monocytes with supernatants of Fc ϵ RI-activated LC-DCs (LC+) induces an increased migration. CTR, Control. Addition of neutralizing mAb to MCP-1 to the supernatant of Fc ϵ RI-stimulated LC-DCs reverses the increased migration of monocytes (Supern. LC-DC+ α MCP-1; $n = 5$). **B**, Chemotactic signals released by LC-DCs and IDEC-DCs induce a higher migration of autologous naive CD4 $^{+}$ CD45RA $^{+}$ (open bars) and memory CD4 $^{+}$ CD45RO $^{+}$ T cells (filled bars; $n = 5$). MCP-1 production of the CD1a $^{+}$ CD11b $^{-}$ LC population from the nonlesional skin of atopic patients after stimulation with Fc ϵ RI is shown (**D**). The respective isotype control is shown on the left side (**C**). This is one representative experiment ($n = 3$).

Statistical analysis

Statistical analysis was performed by using the paired Student t test (SPSS for Windows 8.5). A P value of less than .05 was considered statistically significant.

RESULTS

Characterization of LC-DCs and IDEC-DCs generated in vitro

Detailed phenotypic analysis of ex vivo isolated and in vitro generated LCs and IDECs was performed by using flow cytometry in time kinetic experiments. We found that LC-DCs and IDEC-DCs generated in vitro displayed phenotypic characteristics that were similar to those of LCs and IDECs isolated from skin lesions of patients with AD (Fig 1, A and B).

In addition, IDEC-DCs display a considerably higher surface expression of the costimulatory CD80 and MHC II

molecules and Fc ϵ RII (Fig 1, C). Together, IDEC-DCs and LC-DCs generated in vitro are phenotypically very similar to LCs and IDECs in AD skin lesions in vivo.

Activation of Fc ϵ RI in LC-DCs and IDEC-DCs induces a distinct cytokine and chemokine pattern

Cross-linking of Fc ϵ RI on LC-DCs exclusively induces a further increase of the intracellular amount of IL-8, whereas the intracellular amount of other pro-inflammatory cytokines and chemokines, such as IL-1 α and MIP-1 α /CCL-3, decreases significantly (Fig 2, A). In contrast, engagement of Fc ϵ RI on IDEC-DCs enhances the intracellular amount of IL-1 α and MIP-1 α /CCL-3 (Fig 2, B).

Levels of the macrophage-derived chemokine and the thymus and activation-regulated chemokine (CCL-17),

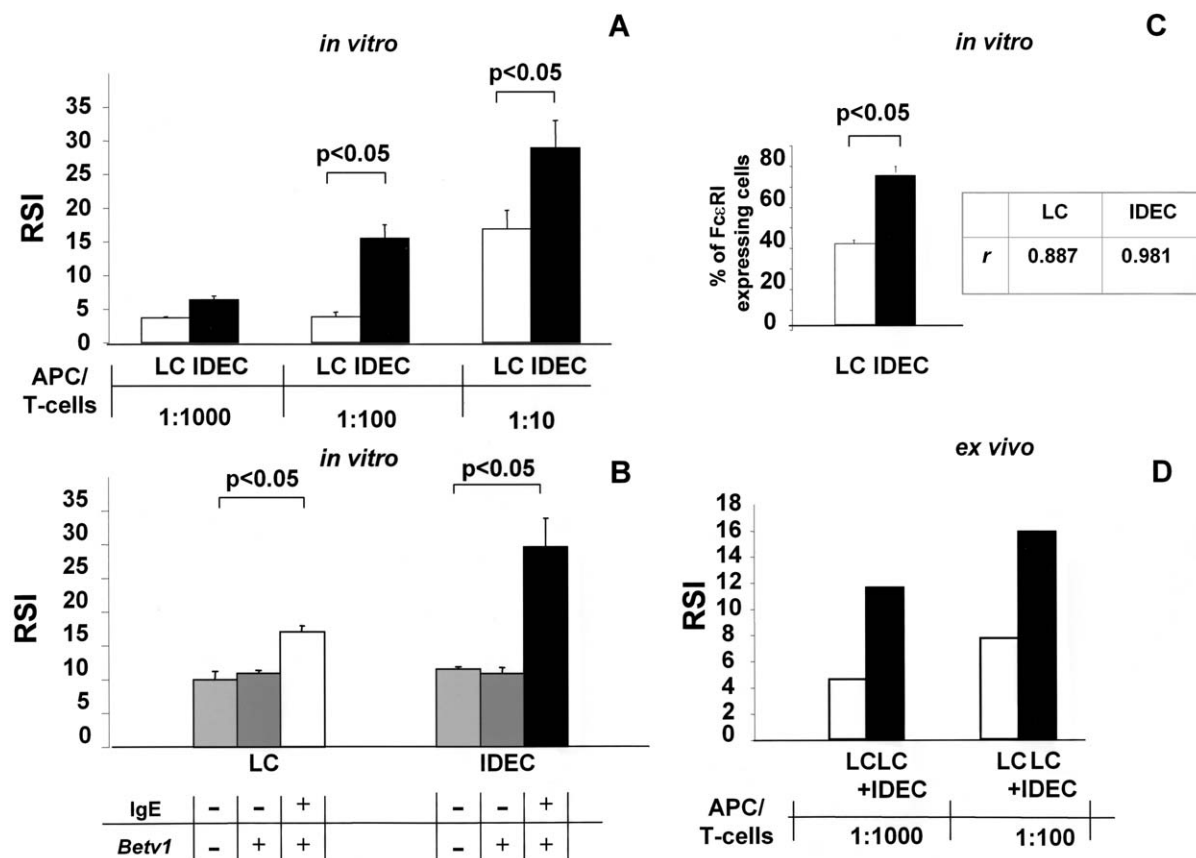


FIG 4. Bet v 1-loaded IDEC-DCs display a higher stimulatory capacity toward Bet v 1-specific TCCs in vitro. **A**, Only LC-DCs and IDEC-DCs preincubated with Bet v 1-specific IgE and Bet v 1 display enhanced stimulatory properties toward allergen-specific TCCs ($n = 5$; **B**). The stimulatory capacity of LC-DCs and IDEC-DCs correlated positively with the FcεRI surface expression (**C**). Epidermal cell suspensions from the lesional skin of patients with AD display a higher stimulatory capacity (filled bars; **D**) than epidermal cell suspensions isolated from nonlesional skin (open bars; **D**). This is one representative experiment ($n = 3$). r = correlation coefficient.

which enable DCs to recruit other cell types, were already high in the supernatants of unstimulated LC-DCs and IDEC-DCs, and no further increment could be obtained by means of FcεRI cross-linking (data not shown). In contrast, a significantly augmented release of the macrophage chemoattractant protein 1 (MCP-1; CCL-2) could be achieved through FcεRI ligation on LC-DCs (Fig 2, C), which produce even higher MCP-1 baseline levels than IDEC-LCs. The amount of IL-16 increased in both LC-DCs and IDEC-DCs as a consequence of FcεRI ligation (Fig 2, D).

These data imply that LCs might play a role in the recruitment of other cells, such as monocytes, CD4⁺ T cells, and CD8⁺ T cells, which invade in high densities into the skin lesions of patients with AD in vivo.

FcεRI-ligated LC-DCs and IDEC-DCs show high recruitment of monocytes and subtypes of T cells in vitro

Monocytes express the chemokine receptors CCR2, CCR4, and CCR6 in high amounts on their cell surface

(Fig 3, A, box). Interestingly, we found that monocytes obtained from the peripheral blood had an increased migratory capacity, which could be induced by incubating the monocytes with supernatants of FcεRI-stimulated LC-DCs (Fig 3, A). As a control, incubation of supernatants of FcεRI-stimulated LC-DCs with a neutralizing antibody to MCP-1 reversed these chemotactic properties of the LC-DCs (Fig 3, A), whereas incubation of the supernatants only did not (data not shown). Incubation with recombinant MCP-1 (CCL-2) was used as a positive control (data not shown). In addition, it should be noted that both cell types, LC-DCs and IDEC-DCs, release soluble mediators capable of attracting naive autologous T cells in migration assays (Fig 3, B). As a proof of concept, we could show that FcεRI/CD1a/CD11b-positive LCs isolated ex vivo from the nonlesional skin of atopic patients display increased intracellular amounts MCP-1 within 6 hours after FcεRI activation (Fig 3, C). These data suggest that MCP-1 produced by LCs might serve as an important chemoattractant for monocytes from the peripheral blood in patients with AD in vivo. In this way LCs might

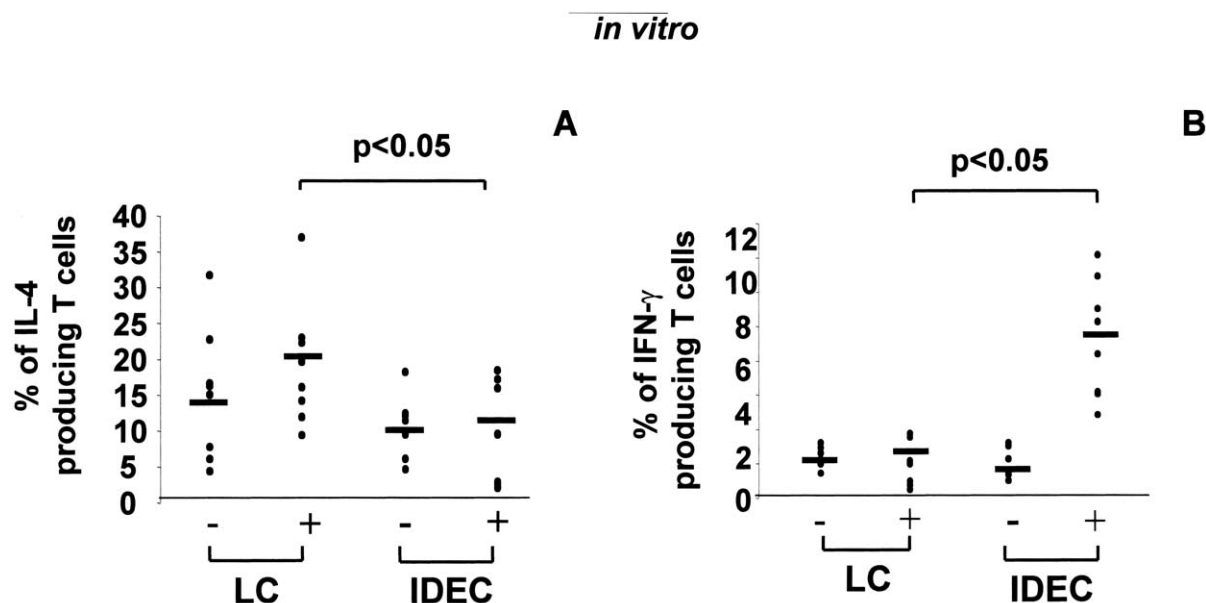


FIG 5. Differential induction of IL-4 and IFN- γ in naive T cells by unstimulated or Bet v 1-loaded IDEC-DCs and LC-DCs in vitro. The percentage of IL-4⁺ (**A**) or IFN- γ ⁺ (**B**) T cells after coculture with irradiated unloaded (–) or IgE/Bet v 1-loaded (+) LC-DCs and IDEC-DCs is depicted.

contribute to the recruitment of precursor cells of DC subtypes, such as IDECs, at LC-rich sites in the peripheral tissues.

IDECs cause potent antigen-specific T-cell proliferation through Fc ϵ RI

Proliferation assays with recombinant specific IgE and specific MCH II-restricted T-cell clones (TCCs) were performed to clarify whether DC subtypes display a heterogeneous antigen-presenting capacity through Fc ϵ RI and stimulatory activity toward antigen-specific T cells.

IDEC-DCs loaded with Bet v 1-specific IgE and recombinant Bet v 1 were significantly better stimulators of Bet v 1-specific TCCs than LC-DCs. A greater T-cell proliferation was obtained with IDEC-DCs compared with LC-DCs at cell ratios of 1:10, 1:100, and 1:1000 antigen-presenting cells/TCCs (Fig 4, A). The control groups comprised unloaded LC-DCs and IDEC-DCs stained with anti-IgE to exclude any constitutive binding of IgE. Furthermore, LC-DCs and IDEC-DCs incubated with Bet v 1 antigen alone and LC-DCs and IDEC-DCs without preincubation with Bet v 1-specific IgE are shown (Fig 4, B). In these experiments no significant increase of the TCC proliferation could be achieved. In contrast, preincubation of the cells with a blocking antibody to Fc ϵ RII (CD23), because IDEC-DCs express Fc ϵ RII, as shown in Fig 1, did not have any influence on the outcome of the T-cell proliferation (data not shown), whereas preincubation with blocking antibody to Fc ϵ RI abrogates the stimulatory capacity of LC-DCs and IDEC-DCs toward TCCs (data not shown). Notably, the antigen-specific stimulatory capacity of IDEC-DCs and LC-DCs toward TCCs

correlated positively with the Fc ϵ RI surface expression of these cells (Fig 4, C). We could also show that epidermal cell suspensions from lesional skin containing IDEC-DCs display a higher stimulatory activity toward autologous T cells than epidermal cell suspensions from nonlesional skin containing exclusively LC-DCs (Fig 4, D). Altogether, these data imply that the IgE-mediated Bet v 1 allergen uptake of LC-DCs and IDEC-DCs is achieved mainly through Fc ϵ RI, and IDEC-DCs might strongly amplify the allergic inflammatory immune response and proliferation of T cells in vivo.

Fc ϵ RI-mediated antigen uptake by LC-DCs and IDEC-DCs leads to a different polarization of naive T cells

The next series of experiments were designed to evaluate whether LC-DCs and IDEC-DCs differ in their capacity to prime autologous naive T cells. For this purpose, the quality and quantity of the typical T_H2 cytokine IL-4 and T_H1 cytokine IFN- γ levels of autologous naive T cells that were cocultured with IgE Bet v 1 allergen complex loaded or unloaded LC-DCs and IDEC-DCs were evaluated in functional assays.

Unloaded LC-DCs and LC-DCs activated through Bet v 1 allergen-IgE immune complexes induced a higher amount of IL-4-producing T cells (Fig 5, A). In contrast, IDEC-DCs preincubated with allergen-IgE immune complexes elicited a greater proportion of T cells producing the T_H1 cytokine IFN- γ than LCs (Fig 5, B).

In summary, these data show that LC-DCs and IDEC-DCs express T cell-polarizing factors that are adaptive to further modulation by soluble and cellular components,

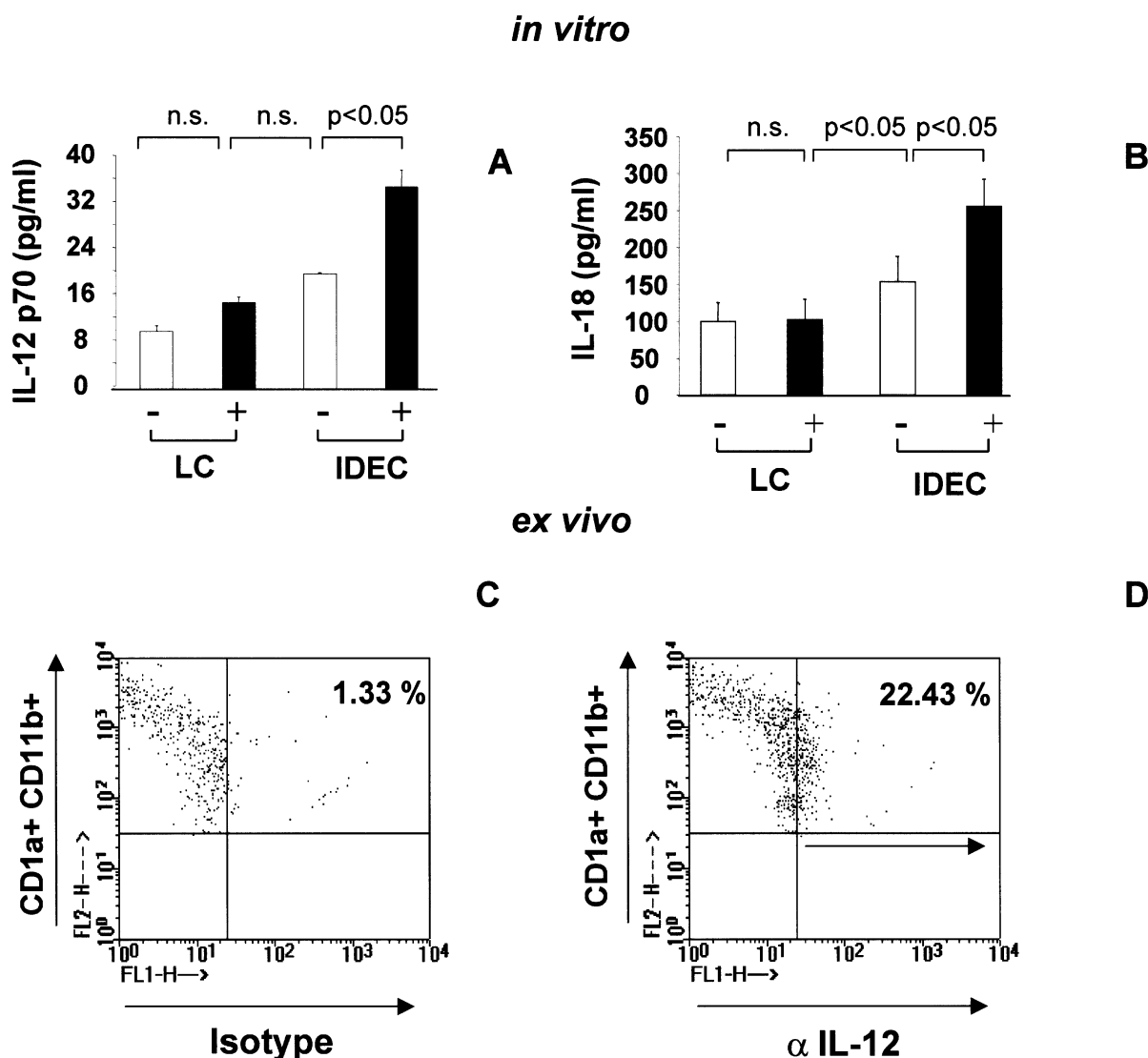


FIG 6. IDECs release the cytokines IL-12 and IL-18 after Fc ϵ RI ligation in vitro. High amounts of IL-12p70 (A) and IL-18 (B) are detectable in the supernatants of Fc ϵ RI-stimulated IDEC-DCs ($n = 8$ experiments). The capability of CD1a⁺ CD11b⁺ IDECs isolated ex vivo to produce IL-12p40/p70 is shown in the top right quadrant (D). Isotype control is shown on the left side (C). *n.s.*, Not significant.

indicating a dynamic regulation of the generation of T_H1 or T_H2 cells during the immune response in vivo.

T_H1 and T_H2 polarization is a stochastic process that is promoted mainly by soluble factors, such as IL-12 and IL-18. On account of this, it was important to evaluate whether Fc ϵ RI engagement of LCs and IDECs would enable them to produce these cytokines in a different manner. Interestingly, IDEC-DCs released IL-12p70 in high amounts after Fc ϵ RI activation. Additionally, Fc ϵ RI-engaged IDEC-DCs had a high potential to produce IL-18, which has been identified as a factor promoting IL-12 and might contribute to the outcome of a T-cell response of the T_H1 type induced by these cells (Fig 6, A and B).

As a proof of concept, we could show that in Fc ϵ RI⁺ CD1a⁺ CD11b⁺ IDECs isolated ex vivo from the lesional

skin of patients with AD, enhanced amounts of IL-12 are detectable after activation with proinflammatory stimuli, such as LPS (Fig 6, C).

DISCUSSION

Our data suggest that a dynamic interaction of Fc ϵ RI⁺ LCs and Fc ϵ RI⁺ IDECs and a distinct capability to prime T cells underlies the biphasic nature of AD. First, Fc ϵ RI engagement of resident LCs in the epidermis contributes to the initiation of the inflammatory response in which T_H2 cytokines predominate. Thereafter, the release of chemokines from Fc ϵ RI-stimulated LCs accounts for the recruitment of IDECs and of T cells into the epidermis.²¹⁻²³

Invading from the peripheral blood, IDECs equipped with high amounts of FcεRI on their cell surfaces are capable of accelerating the turnover rate of the antigen uptake, and as a consequence of the intensive FcεRI triggering, IDECs augment the amount of proinflammatory cytokines and chemokines released, thereby serving as strong amplifiers of the ongoing allergic immune response. The mechanisms described here are specific processes mediated through allergens and allergen-specific IgE on the surface of LCs and IDECs in vitro and in vivo. Allergen presentation by epidermal LCs from patients with AD to T cells has been shown to be mediated through allergen-specific IgE molecules in previous studies.²⁴

Furthermore, the capacity of IDEC-DCs to release both cytokines IL-12 and IL-18 in high amounts after FcεRI cross-linking or after activation with proinflammatory stimuli, such as LPS, sustains the development of an immune reaction of the T_H1 type.²⁵ In this way IDECs might contribute to the switch from the initial phase of AD into the IFN-γ-predominant chronic eczematous phase. The polarization of both naive and memory T cells and the induction of keratinocyte apoptosis, which is a characteristic feature of eczematous skin diseases, such as AD, is a flexible interplay of allergens, soluble factors, and proinflammatory signals of the microenvironment.²⁶ In addition, a reciprocal cross-talk between LCs, IDECs, T cells, and other cell types, such as keratinocytes, in the epidermal skin lesions through the release of chemokines by LCs and IDECs might contribute to the recruitment of more DC precursors and T-cell subtypes and produce, in this way, the vicious circle of the inflammatory reaction.^{27,28}

Thus far, the cellular source of IL-12 in the skin lesions of patients with AD was unknown, even though eosinophils have been believed to display relevant IL-12 production. In this study we provide evidence that FcεRI-stimulated IDECs might represent another cellular source of IL-12. This hypothesis is consistent with the observation that after successful topical treatment, the number of IDECs in the epidermal skin lesions of patients with AD decreases to less than the detectable level.²⁹ In addition, a profound decrease of IL-12 could be observed in the successfully treated skin of patients with AD.^{24,30,31} Because keratinocytes coexpress IL-18 at the mRNA level, as well as at the protein level, they might represent an important additional source of IL-12 and IL-18 in vivo.³² In contrast to recent concepts explaining the polarization process of DCs, both LC and IDEC subtypes develop from the same precursor monocyte, and the influence of additional soluble and membrane-bound signals leads to the outcome of the T_H1 or T_H2 cytokine response of T cells.³³ Kapsenberg et al promote the concept that DCs priming naive T cells, in addition to signals mediated through MHC peptide complexes and costimulatory molecules, carry a third signal that could be mediated by soluble and membrane-bound molecules that promote the generation of type 1 effector DCs (DC1), producing high amounts of IL-12 or the generation of IL-12-deficient DC2, which drives the development of T_H2

cells. Considering this hypothesis, IDECs would have DC1 properties, whereas LCs would resemble the DC2.³⁴

Taken together in the light of these data, a scenario emerges that both LCs and IDECs have a crucial position in the complex pathophysiologic network of AD. In support of the latter, we provide clear evidence that LCs and IDECs contribute distinctly to the outcome of T-cell responses, which determine the clinical course and the biphasic nature of AD.

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